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Forward Stepping Mechanism of Kinesin-1 Studied using Asymmetrically-Joined Two-Headed Monomer
Hiroshi Isojima, Michio Tomishige.
Kinesin-1 moves processively along microtubule by alternately moving two motor domains, but the mechanism of the preferential forward stepping is still controversial. The ''neck linker-docking model'' proposes that the neck linker docking of the microtubule-bound head generates forward bias of the tethered head. However, our recent structural analysis of kinesin dimer (Makino et al.) suggested an alternate model in which the tethered head position does not necessarily be biased because the tethered head is not allowed to bind to the rear tubulin-binding site due to a steric constraint on its neck linker and can only release ADP at the forward binding site (''biased-binding model''). To distinguish these mechanisms as alternate steps, we engineered two-headed monomer kinesin by joining two motor heads in tandem on a single polypeptide, in which the neck linker of first head (N-head) is connected to second head (C-head) so that it can propel C-head forward, whereas the neck linker of C-head is free. Single molecule fluorescence observation showed that this two-headed monomer moves processively along microtubules although the velocity was smaller than wild-type dimer by four-fold. In addition, FIONA measurement of individual head showed that both heads takes discrete 16 nm steps, illustrating that this monomer moves by alternately exchanging two heads. Then we measured the dwell time of alternate steps using single molecule FRET and found that forward-stepping of C-head presumably driven by the neck linker docking was less efficient than the forward-stepping of N-head, because the tethered C-head often rebinds to the rear-binding site. These results suggest that biased-binding mechanism is more efficient to drive forward stepping, because rebinding of the tethered head to the rear-binding site is effectively prohibited.

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Ca^{2+} Dependent Dimerization of Kinesin-CaM and Kinesin-M13 Fusion Proteins
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Kinesin is known as a dimeric motor protein, which carries cellular cargoes along microtubules by hydrolyzing ATP. Calmodulin (CaM) is a calcium binding protein that participates in cellular regulatory processes. CaM undergoes a conformational change upon binding to calcium, which enables it to bind to specific proteins for a specific response. We have previously demonstrated that kinesin fused with CaM at the C-terminal binds reversibly to M13-Qdots in a calcium dependent manner. In this study, we tried to make the calcium dependent reversible dimerization of kinesin utilizing CaM- target peptide M13 binding system in order to control motility of kinesin. First we designed and prepared the cDNA of the truncated kinesin (355 amino acids) that does not form dimer. We prepared the cDNA encoding two kinesin chimeric proteins in which C-terminal of kinesin355 was fused with calmodulin (K355-CaM) and fused with M13-Cys (K355-M13-cys). The cDNAs of the kinesin chimeras were cloned into expression vector pET21a and transformed into E.coli BL21 (DE3). The kinesin chimeras were successfully expressed and purified by Co-Chelate column. These kinesin chimeras showed normal ATPase activities. Furthermore, K355-CaM bound to M13-YFP in a calcium dependent manner. The calcium dependent interaction between K355-CaM and K355-M13-Cys was examined using Size-exclusion chromatography (SEC)-HPLC, whereas two chimeras did not bind in the absence of Ca^{2+}. In vitro motility assay demonstrated that the dimerized chimera induced microtubule gliding in the presence of Ca^{2+}.

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The Kinesin-1 C-Terminal Tail is Intrinsically Disordered
Mark Seeger, Yongbo Zhang, Sarah Rice.
The C-terminus of kineisn-1 has long been referred to in the literature as the globular tail. In this work we show that this domain is in fact intrinsically disordered. The unfolded structure of the tail domain is revealed via in silico prediction methods, and CD and NMR spectroscopies. It has been well established that a diverse collection of cargos bind exclusively to the tail domain of kinesin-1, and being natively unstructured would allow the tail to sample a variety of conformations in order to accommodate these various binding-partners. Expanding the in silico methods to include other kinesins, we predict that the cargo-binding domains of most members of the human kinesin superfamily are disordered to varying degrees. Therefore, intrinsically disordered sequences may be a general mechanism of cargo binding for many kinesin heavy chains.

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KIF1A Repeats Cycle of 'FREE Diffusion' and 'SPECIFIC Binding' during Weak Binding State
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The nature of intermolecular interaction between motor and cytoskeletal filament during the weak binding state is not fully understood. In the case of kinesin, while structural analyses revealed that kinesin binds to a specific binding site on tubulin, motility data suggested that kinesin undergoes diffusion, searching for its next binding site. To understand how specific binding and diffusion are compatible in a single ADP state, we analyzed the motion of the single-headed kinesin KIF1A on various mutant microtubules (MTs) in the presence of ADP, using the single molecule motility assay.
We prepared two series of mutant MTs. The first is a series with increased/decreased negative charges at the C-terminal tails (CTTs) of tubulin, reported to be indispensable for the weak binding of KIF1A to the MT (Okada et al., 2000). The second is a series of charged-to-alanine mutants in the H11-12 loop and H12 of tubulin (α-E415, -E416, -E418, -E421 and β-E410, -D417), found to be critical for kinesin motility and ATPase (Uchimura et al., 2010). The analyses of KIF1A movement showed that a reduction of negative charges in CTTs leads to a reduction in both the duration of interaction and the diffusion length of KIF1A, yet the diffusion constant was not greatly changed. In contrast, in most of the charged-to-alanine tubulin mutants, the diffusion constant of KIF1A increased and the duration shortened, but the diffusion length was unaffected. These results indicate that KIF1A-MT interaction in the ADP state can be modeled as an equilibrium between two substates: a dynamic 'diffusion state' and a static 'binding state'. While CTTs stabilize the former, the critical residues in the H11-12 loop and H12 of tubulin stabilize the latter. This model is applicable to dimeric kinesin.

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Mechanochemical Properties of the Kinesin-2 Motor, KIF3A/B, Studied by Optical Trapping
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The kinesin-2 motor KIF3A/B is a processive transport motor that incorporates two different motor domains, coded by separate KIF3A and KIF3B polypeptides. In intraflagellar transport, kinesin-2 motors transport cargo towards the tips of cilia, and dynein motors attached to the cargo are responsible for transport back towards the cell body. While it is known that these opposing motors are responsible for bidirectional transport, little is known about the performance of kinesin-2 motors under load. Here, we used a feedback-controlled optical trap to probe the nanomechanical properties of full-length mouse KIF3A/B under various load regimes and nucleotide concentrations. In addition, each motor domain was characterized by studying mutants consisting of two identical motor domains. Compared to conventional kinesin-1, kinesin-2 velocities were less dependent on load. Moreover, motor processivity, as measured by the run length, depended strongly upon the external load. In a tug-of-war with dynein, such characteristics are expected to enhance the dynamics of directional switching during transport, compared with kinesin-1 which slows under load but remains processive. Experiments using chimeric motors indicate that the load-dependent properties of kinesin-2 are attributable to their motor domains and not, for example, to the lengths of the neck linkers, nor to the properties of the coiled-coil stalks. The velocity data can be modeled in terms of twin alternating three-state cycles, one for each type of motor domain, where ATP binding is followed by a load-dependent transition, presumably neck-linker docking, before hydrolysis. Modeling also suggests that neck-linker docking represents a key mechanochemical step with a shorter characteristic distance for kinesin-2 than kinesin-1. A reduced characteristic distance may facilitate hydrolysis under load and reduce the probability that the tethered motor domain reaches the next microtubule binding site, leading to diminished processivity.

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Neck-Linker-Length Dependence of Processive Kinesin-5 Motility
André Düselder, Christina Thiede, Stefanie Kramer, Christoph F. Schmidt, Stefan Lakämper.
To explore the basic motor activity of the mitotic Kinesin-5, we previously constructed a stable dimeric Kinesin-5 head/Kinesin-1 stalk chimera (Eg5Kin), which contains the motor domain and 14 amino acids of the neck linker of Xenopus leavis Eg5 fused to the neck coiled coil of Drosophila melanogaster Kinesin-1. In contrast to truncated dimeric Eg5-513 (Valentine and Block, 2009, Biophys. J. 97:1671), Eg5Kin is a highly processive motor (Lakämper et al., 2010, J. Mol. Biol. 399:1).